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Association between serum neopterin, obesity and daytime sleepiness in patients with obstructive sleep apnea

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Summary

Objective: Obesity and obstructive sleep apnea (OSA) and systemic inflammation may interact through biochemical pathways. Neopterin (NP) is a monocyte/macrophage activation marker produced by macrophages in response to interferon-gamma secreted by activated T-lymphocytes. This study examines the association between NP, obesity and OSA.

Patients and methods: The study included 22 newly diagnosed OSA (+) patients and 18 OSA (–) patients. Subjects with history of coronary artery disease, transplant patients, history of alcohol and drug abuse, history of HIV and any other significant medical illnesses such as active infections, autoimmune disease, malignancy, liver disease, pulmonary disease (COPD, asthma,...), neuromuscular disease, patients on immunomodulating therapy or HMG-CoA reductase inhibitors were excluded.

Results: There were no significant differences in age, body mass index (BMI), and smoking habits of the OSA (+) patients and OSA (–) patients. Serum NP levels did not show any significant difference between the OSA (+) patients and OSA (–) patients, however, NP levels were positively correlated with BMI ($r = 0.320$, $p = 0.044$). There was no significant correlation between NP and any of the polysomnographic parameters. The result of stepwise regression analyses ($r^2 = 0.320$, $p < 0.001$) showed that high serum NP levels ($p = 0.004$) and apnea–hypopnea index (AHI) were a risk factor for elevated Epworth sleepiness score, independent of BMI.

Conclusion: We suggest that serum NP levels correlate with BMI. There was a significant relationship between serum NP levels and excessive daytime sleepiness in OSA patients.

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Introduction

Obstructive sleep apnea (OSA) is characterized by repetitive collapse of the upper airway during sleep, resulting in obstruction of airflow and oxygen desaturation, which cause arousal from sleep.¹ OSA is a common disorder in the adult population, strongly associated with obesity. Obesity is a major public health problem. There is a causal relationship between OSA and obesity. OSA is present in approximately 40% of obese individuals, and approximately 70% of OSA patients are obese.²

Several studies have demonstrated that OSA may be one of the most important risk factors for cardiovascular disease (CVD).^{3,4} The exact mechanism of cardiovascular disease development in patients with OSA is unknown. Several possible mechanisms such as increased sympathetic activation, increased oxidative stress, increased platelet aggregation, metabolic dysregulation, inflammation, and systemic vascular endothelial dysfunction are suggested.⁵ In addition, adipocytes can produce inflammatory cytokines. There is also a relationship between obesity, cardiovascular risk and inflammation.⁶ Neopterin (NP) is an aromatic pteridine and a by-product of the guanosine triphosphate bipterine pathway. NP is produced by macrophages that are stimulated by interferon-gamma (IFN-gamma) secreted by activated T-lymphocytes.^{7,8} Some studies have shown that elevated serum NP level may be used as a monocyte/macrophage activation marker.^{9,10}

In the present study, we hypothesized that OSA and/or obesity may elevate NP. In order to test this hypothesis, we compared serum NP levels between OSA patients and nonapneic controls.

Materials and methods

Subjects

A cross-sectional study was performed, including subjects referred to the same sleep center for the evaluation of suspected OSA. The study was planned according to the ethics guidelines of the Helsinki Declaration and was approved by the Institutional Research Ethics Board of Uludağ University Medical Hospital, Bursa, Turkey. All patients gave written informed consent regarding participation in this study. The exclusion criteria were history of coronary artery disease, transplant patients, history of alcohol and drug abuse, history of HIV and any other significant medical illnesses such as active infections, autoimmune disease, malignancy, liver disease, pulmonary disease (COPD, asthma,...), neuromuscular disease, being on immunomodulating therapy and use of HMG-CoA reductase inhibitors.

The questionnaire inquired about the presence of any history of snoring, witnessed apnea and Epworth Sleepiness Scale (ESS).¹¹ Demographic information (age, gender, and smoking habits) and anthropometric measurements (height, weight, and BMI [body mass index: weight/height {kg/m²}] were obtained upon presentation to the sleep center.

Sleep study

Full polysomnography (PSG) monitoring was performed in all patients using the Compumedics P-series Sleep System (Compumedics Sleep, Melbourne, Australia). Polysomnographic recordings were done with standard technique. Sleep staging was performed according to the standard criteria of Rechtschaffen and Kales.¹² The sum of time spent in apnea and hypopnea was divided by the total sleep time to obtain the apnea–hypopnea index (AHI). Subjects with AHI ≥ 5 were considered to have OSA (+). Subjects with AHI < 5 were categorized as the OSA (–) patients.

Determination of NP levels

Venous blood was collected from all patients between 08:00 am and 09:00 am after the sleep study and centrifuged at 4 °C, 3000×g for 10 min within 30 min of collection. Serum samples were stored at –80 °C until assessment was carried out in batches at the end of recruitment. Serum NP levels were determined by a commercially available enzyme immunoassay kit (Neopterin EIA kit, ARP, Belmont, USA) according to the manufacturer's instructions. Briefly, samples were placed into wells coated with a polyclonal antibody having a high affinity for neopterin and horseradish peroxidase conjugated antibody was added for detection. After 2-h incubation at room temperature, excess unbound conjugate was removed by washing. Then color substrate (TMB) was placed into the wells. Absorbance was measured in a microplate reader at 450 nm. The concentration of the NP was expressed as nanogram per milliliter (ng/ml) as recommended in the kit. The expected physiological ranges were 0.3–3.0 ng/ml.

Statistical analysis

Statistical analysis was performed using the SPSS package for Windows, version 13.0. Comparisons of data between OSA (+) and OSA (–) patients were carried out by Student's *t*, Chi-Square and Mann–Whitney *U* tests. Concordance of normal distribution of all variables was calculated by Shapiro–Wilk test before comparison between OSA (+) and OSA (–) patients. If the data were not normally distributed, we used non-parametric tests for dependent variables. The relationship between serum NP level and BMI was calculated using Pearson's correlation analysis. We performed stepwise regression analysis to identify significant relationships between BMI, sleep disorder parameters and NP. *p* value less than 0.05 was considered statistically significant.

Results

Baseline characteristics

Sixty-five subjects with suspected OSA were screened for the study. Twenty-five subjects were excluded. The study included 22 consecutive newly diagnosed OSA (+) patients and 18 OSA (–) patients. Baseline clinical and polysomnographic characteristics of the OSA (+) and OSA (–) patients are shown in Table 1. There was no significant difference in

Table 1 Baseline clinical and polysomnographic characteristics of the study groups

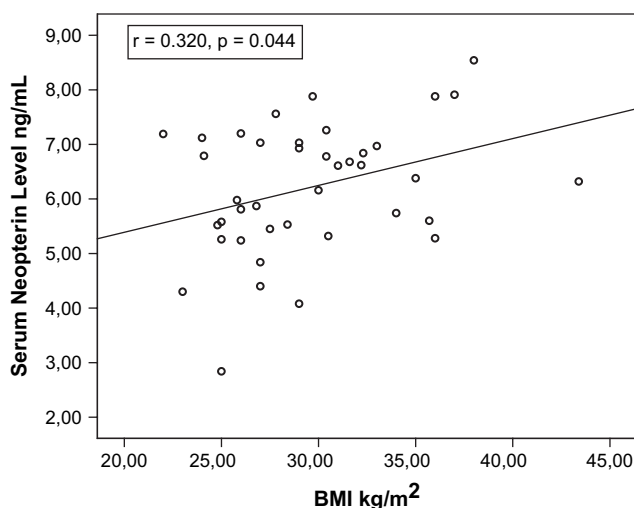
	OSA (+)	OSA (–)	<i>p</i> value
Age	47.0 ± 2.9	48.5 ± 2.1	NS
Body mass index (kg/m ²)	30.1 ± 1.1	28.3 ± 1.9	NS
Smoking habits (pack-years)	23.1 ± 6.9	19.2 ± 2.7	NS
Epworth sleepiness scale	8.4 ± 1.1	4.6 ± 0.8	<0.05
Total sleep time (TST) (h)	6.3 ± 0.7	6.5 ± 0.9	NS
Sleep efficiency (%)	81.3 ± 2.2	81.1 ± 1.9	NS
Stages 3 and 4 (% TST)	8.1 ± 1.8	16.8 ± 1.8	<0.05
Rapid eye movement (% TST)	11.1 ± 1.6	20.3 ± 1.7	<0.05
AHI (per h)	47.0 ± 4.6	2.1 ± 0.3	–
Arousal (per h)	26.8 ± 3.5	12.4 ± 1.9	<0.05
Baseline oxygen saturation (%)	93.7 ± 2.5	94.3 ± 1.1	NS

NS: statistically insignificant.

age, gender, BMI, smoking habit, and snoring between the two groups. In the OSA (+) group, ESS scores were significantly higher than those of the OSA (–) patients ($p < 0.05$). There was no significant difference in the total sleeping time, sleep efficiency, and baseline oxygen saturation between the two groups. Significant differences were noted in the sleep stages 3 and 4, number of arousals (per hour), AHI, duration of apnea–hypopnea, oxygen desaturation index, average oxygen saturation during sleep, average oxygen desaturation, and length of time spent with an oxygen saturation <90% when the OSA (+) group was compared to OSA (–) patients ($p < 0.001$).

Serum NP levels

No significant difference was noted in the serum levels of NP in OSA (+) group (6.4 ± 0.2 ng/ml) when compared to OSA (–) patients (6.0 ± 0.3 ng/ml). Serum NP levels were positively correlated with BMI ($r = 0.320$, $p = 0.044$)

**Figure 1** Correlation coefficient between serum NP levels and body mass index (BMI).

(Fig. 1). There was no significant correlation between NP and any polysomnographic parameters such as apnea–hypopnea index (AHI), number of total apnea during the night, number of respiratory events during the night, time spent in apnea and hypopnea, respiratory arousal index, average oxygen desaturation and oxygen desaturation index.

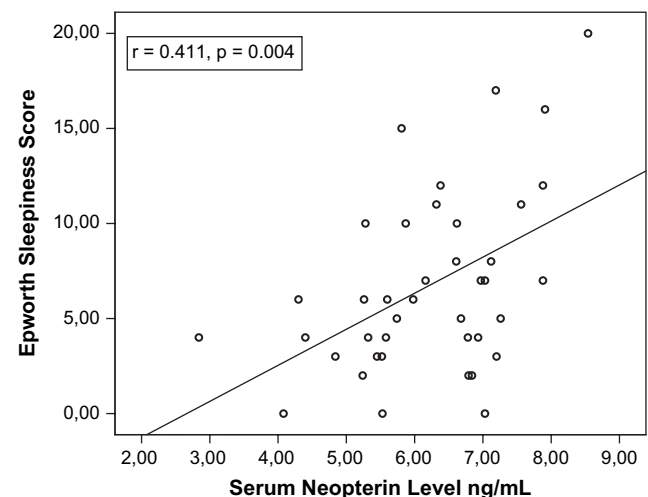
The result of stepwise regression analyses [ESS = NP (0.411) + AHI (0.367) + BMI (0.141), $r^2 = 0.320$, $p < 0.001$] showed that NP ($p = 0.004$) (Fig. 2) and AHI ($p = 0.009$) were risk factors for elevated ESS, independent of BMI.

Discussion

To our knowledge this is the first study to assess the relationship between serum NP levels, obesity and OSA. The results of the present study indicate that there is a significant relationship between excessive daytime sleepiness, obesity and elevated serum NP levels.

NP is a sensitive marker of cellular immunity. Activated lymphocytes produce IFN-gamma, which in turn activates macrophages. Macrophages activated by IFN-gamma synthesize NP. NP modulates the intracellular redox state and it activates translocation of the nuclear factor κ B subunits to the nucleus. NP is upregulated by proinflammatory genes.^{13,14} NP may be of clinical use as an early inflammation marker. Elevated NP concentrations are observed in diseases with intensified monocyte/macrophage activity. The NP concentrations also reflect the level of oxidative stress caused by activation of the immune system.¹⁵

Inflammation plays an important role in atherogenesis and plaque vulnerability. Monocytes/macrophages are culprit cells in atherosclerotic plaque formation. Studies have reported an association between NP and atherosclerosis.^{16–19} Activated lymphocytes in atherosclerotic plaques produce a variety of cytokines such as IFN-gamma, a molecule activating macrophages. The levels of NP are elevated in patients with acute as well as chronic coronary artery disease (CAD).¹⁷ In addition, serum NP levels in patients with acute myocardial infarction (AMI) were significantly higher than chronic CAD and control subjects. NP is a marker of CAD

**Figure 2** Regression line between Epworth sleepiness scale and serum NP levels.

activity and it is a prognostic factor for acute coronary syndrome (ACS).¹⁸

Morphological thickness of the arterial wall is an important feature of atherosclerosis. Carotid arterial changes can reflect the progression of systemic atherosclerosis in asymptomatic general population. Erten et al.²⁰ reported a significant positive correlation between serum NP levels and carotid intima media thickness (IMT) in hemodialysis patients. They suggested that NP could be associated with the severity of carotid atherosclerosis. Previous studies showed that the carotid IMT is increased in patients with obesity and/or severe OSA.^{21,22}

Increasing evidence indicates the important role of inflammation in the etiology of major public health problems. Several studies have proposed that obesity might be an inflammatory disorder. Obesity is closely associated with a number of established cardiovascular risk factors, including diabetes mellitus, insulin resistance, dyslipidemia, CAD, and hypertension.²³ Adipocytes can produce inflammatory cytokines. Adipose tissue is known to express and secrete a variety of products known as 'adipokines', including leptin, adiponectin, resistin and visfatin, as well as cytokines and chemokines such as tumor necrosis factor- α , interleukin-6 and monocyte chemoattractant protein-1.²⁴ Recent data demonstrate that obese adipose tissue is infiltrated by macrophages, which may be a major source of locally-produced proinflammatory cytokines. Interestingly, weight loss is associated with a reduction in the macrophage infiltration of adipose tissue and an improvement of the inflammatory profile of gene expression. Several factors derived not only from adipocytes but also from infiltrated macrophages probably contribute to the pathogenesis of insulin resistance.²⁵ Ledochowski et al.²⁶ reported that NP concentrations were significantly higher in patients with elevated BMI and elevated glucose concentrations. They also indicated that there were significant correlations between glucose concentrations and BMI, as well as between serum NP concentrations and BMI. Bozdemir et al.²⁷ investigated serum C-reactive protein (CRP) and NP levels in healthy adults. They demonstrated that serum NP levels were higher in group A (BMI ≥ 25 kg/m²) than in group B (BMI < 25 kg/m²), but the differences were not significant. Serum CRP and NP levels were significantly higher in group C (waist-hip ratio, WHR ≥ 0.9) compared to those in group D (WHR < 0.9). In the present study we showed that consistent with previous studies in obese patients serum NP levels were positively correlated with BMI.

Obesity can be associated with excessive daytime sleepiness (EDS) even in the absence of OSA. EDS is considered to be the major sign of OSA, however, the relationship between EDS and AHI has been shown to be weak.²⁸ Bixler et al.²⁹ suggest that patients with a complaint of EDS should be thoroughly assessed for depression, obesity/diabetes and metabolic syndrome independent of whether or not sleep disordered breathing is present. Recent studies suggested that EDS may be more strongly associated with obesity and inflammatory markers than to AHI.^{30–32} In the present study, the result of stepwise regression analyses showed that ESS was a risk factor for high serum NP levels, independent of AHI and BMI.

In conclusion, we suggest that serum NP levels correlate with BMI. There was a significant relationship between serum NP levels and EDS in OSA patients.

Conflict of interest statement

There is no conflict of interest.

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